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Review
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Treatment of large bone defects in load-bearing bone: traditional and novel bone grafts

Dan YU[1,2*], Wenyi SHEN[1,2*], Jiahui DAI[1,2], Huiyong ZHU[1,2✉]

[1]*Department of Oral and Maxillofacial Surgery, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou 310003, China*
[2]*Department of Stomatology, Zhejiang University School of Medicine, Hangzhou 310058, China*

Abstract: Large bone defects in load-bearing bone can result from tumor resection, osteomyelitis, trauma, and other factors. Although bone has the intrinsic potential to self-repair and regenerate, the repair of large bone defects which exceed a certain critical size remains a substantial clinical challenge. Traditionally, repair methods involve using autologous or allogeneic bone tissue to replace the lost bone tissue at defect sites, and autogenous bone grafting remains the "gold standard" treatment. However, the application of traditional bone grafts is limited by drawbacks such as the quantity of extractable bone, donor-site morbidities, and the risk of rejection. In recent years, the clinical demand for alternatives to traditional bone grafts has promoted the development of novel bone-grafting substitutes. In addition to osteoconductivity and osteoinductivity, optimal mechanical properties have recently been the focus of efforts to improve the treatment success of novel bone-grafting alternatives in load-bearing bone defects, but most biomaterial synthetic scaffolds cannot provide sufficient mechanical strength. A fundamental challenge is to find an appropriate balance between mechanical and tissue-regeneration requirements. In this review, the use of traditional bone grafts in load-bearing bone defects, as well as their advantages and disadvantages, is summarized and reviewed. Furthermore, we highlight recent development strategies for novel bone grafts appropriate for load-bearing bone defects based on substance, structural, and functional bionics to provide ideas and directions for future research.

Key words: Bone graft; Bone scaffold; Biomaterial; Load-bearing bone defect; Osseointegration; Osteoconductivity

1 Introduction

The reconstruction of load-bearing bone defects is still a clinical challenge in orthopedics. Bones have strong regenerative capabilities that enable self-repair and regeneration after bone loss. Small bone defects can be repaired by this intrinsic regeneration potential. However, in the case of large bone defects, bone loses its ability to repair itself without external intervention. This may lead to delayed bone union, non-union, and a high rate of patient disability (He et al., 2016; de Grado et al., 2018). Critical-size bone defects (CSDs) are defined as minimum-sized bone defects that cannot heal spontaneously under physiological conditions (Cooke et al., 2020; Linder et al., 2020). Schmitz and Hollinger (1986) initially defined a CSD as the smallest bone defect that could not heal spontaneously without therapeutic intervention. Some scholars have also defined CSDs as the smallest bone defects in an animal that show less than 10% bone regeneration over the animal's lifetime (Hollinger and Kleinschmidt, 1990) or that fail to heal spontaneously during the observation period (Gosain et al., 2000). It is generally believed that CSDs in load-bearing tubular bones should exceed the diameters of long bones by 1.5–2.5 times or occupy over 10% of their lengths (Roddy et al., 2018; Wang et al., 2021). Such defects, not only produce deformities that influence aesthetics and motor function, but also substantially impact quality of life and mental health of a patient.

The question of how to repair CSDs in load-bearing bone and effectively restore function and appearance has become an increasingly attractive research topic. Traditional bone grafting for CSDs in load-bearing bone uses natural bone tissue, including autologous and allogeneic bone grafts (García-Gareta et al., 2015;

✉ Huiyong ZHU, zhuhuiyong@zju.edu.cn
* The two authors contributed equally to this work
Huiyong ZHU, https://orcid.org/0000-0003-0883-5355

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Dou et al., 2016). With the development of materials science, and to overcome the limitations of traditional natural grafts (such as extra trauma, limited supply, and frequent donor site morbidity), the novel method of "occupying the defect" with biological materials instead of natural bone has gradually moved from the laboratory to clinical application. Bone-grafting materials are biomaterials that provide osteoconductive, osteoinductive, and osteogenic properties, and promote bone healing, either alone or in combination with other materials (Bauer and Muschler, 2000). A variety of biomaterials have been designed and are expected to replace traditional bone-grafting materials, providing new therapeutic strategies for the bone defect treatment. There has been a wide discussion of the advantages and disadvantages of the traditional and novel bone grafts available for load-bearing bone defects, which is summarized in Table 1 (Zhang et al., 2020; Stahl and Yang, 2021). In contrast to bone grafts used in non-load-bearing areas, those employed for repairing load-bearing bone defects require greater mechanical performance, which has been a pivotal factor in the development of bone-grafting substitutes for this purpose. Some scholars have recognized the significance of mechanical properties of bone grafts in repairing load-bearing bone defects, and several reviews have been systematically summarized the efforts to determine the affecting factors and improve methods (Ansari et al., 2022; Miri et al., 2024). In this review, we aim to guide readers towards a thorough understanding of the development of bone grafts for load-bearing bone defects from a fresh perspective, rather than solely focusing on the classification of grafting materials. We present traditional and novel bone grafts available for load-bearing bone defects and focus on recent design strategies for promoting bone regeneration based on substance, structural, and functional bionics.

Table 1 Advantages and disadvantages of traditional natural bone grafts and novel artificial bone grafts

Bone-grafting materials	Advantages	Disadvantages
Autografts	• Good osteogenicity, osteoconductivity, and osteoinduction; • Good biocompatibility and no rejection reactions; • Providing essential characteristics for bone regeneration (such as osteoprogenitor cells, osteogenic cytokines, and ideal three-dimensional structural space); • Vascularized autografts can supply the immediate demand for blood and provide sufficient oxygen and nutrients.	• Donor-site morbidity; • Limited availability; • Extra blood loss and pain; • Risk of infection; • Difficult to adapt precisely to the complicated shape of the bone defect.
Allografts	• Good bone conduction; • Convenience; • A relatively abundant source; • Providing comparable structure support and mechanical properties.	• Risk of disease transmission; • Immune rejection; • Limited osteoinductivity.
Novel artificial grafts	• Extensive sources; • Low antigenicity; • Good processability; • Controllable structure, porosity, properties, and properties; • Bioceramics: good osteoinductivity; • Composite materials can maximize the advantages of various materials and improve the comprehensive performance; • Designing personal grafts for the desired shape and mechanical properties according to the requirements of the bone-defect site; • Using scaffold materials with the addition of seed cells and growth factors to promote bone regeneration.	• Metal materials: stress shielding, bio-inertness and potential harmful responses caused by release of metal ions or shedding of metal particles; • Bioceramics: low fracture toughness and brittleness; • Weak mechanical properties of natural polymers and biocompatibility of synthetic polymers.

2 Ideal bone-grafting materials

2.1 Natural bone biology

The ideal bone-grafting materials should mimic the bone tissue composition and structure, as well as the normal course of bone healing, to successfully simulate the healing process of natural bone. Bone tissue is a highly specialized polyporous complex composed of an organic matrix (mainly type I collagen) and inorganic minerals (mainly calcium phosphate). Natural bone tissue is generally categorized into two micro-structural types: cortical (compact) bone, which has a compact arrangement, and cancellous (trabecular or spongy) bone, which has a porous arrangement. Cortical bone, with a porosity ranging from 5% to 15%, consists of osteons and numerous Haversian canals, which serve as conduits for nerves and blood vessels, and form an intricate network (Morgan et al., 2018). Cancellous bone has a loose honeycomb (HC) structure and a porosity of 40%–95%, and accounts for approximately 20% of total bone mass (Morgan et al., 2018). Among other things, the mechanical properties of bone tissue depend on bone composition, porosity, microstructure, and the direction of collagen fibers, which explains why there are significant differences in the mechanical properties between cortical bone and cancellous bone (Martin, 1991). The mechanical properties of cortical bone and cancellous bone are summarized in Table 2 (Henkel et al., 2013).

2.2 Bone defect healing process

Bone healing is a complex and dynamic process consisting of several successive and partially overlapping stages: inflammation, granulation tissue formation, soft and hard callus formation, and bone remodeling (Tansik et al., 2016; Winkler et al., 2018). Each stage involves the formation of a different type of tissue and an interlinked set of cellular events. In the early stages following bone injury, hematoma formation and inflammation in the defect area initiate the healing process. The hematoma fills the void and provides a cellular chemotactic signal that promotes cell migration and directs cell fate. It also provides the initial extracellular matrix (ECM) environment for bone regeneration. Stimulated by the inflammatory response, granulation tissue gradually replaces the hematoma, forming a structured ECM that provides the basis for subsequent bone regeneration. Mesenchymal stem cells (MSCs) are recruited and induced to differentiate into chondrocytes. Subsequently, the granulation tissue gradually transforms into a soft callus dominated by cartilage, providing enhanced but temporary mechanical support and stability. As chondrocytes become hypertrophic, mineralization and vascular invasion occur in the cartilaginous callus, which acts as the template for hard callus. This stage involves resorption of mineralized cartilage by osteoclasts and bone formation by osteoblasts, which transforms the soft callus into a hard callus consisting of immature woven bone tissue. With the action of the osteoblasts and osteoclasts, the immature hard callus is gradually converted into mature lamellar bone through remodeling, yielding bone that should structurally and mechanically match the normal bone tissue. Angiogenesis and osteogenesis occur sequentially in the healing of bone defects and promote the effect of each other.

2.3 Ideal properties of bone-grafting materials

Bone-grafting materials should match as closely as possible the structure, morphology, and mechanical properties of the natural bone at the defect site to create an environment conducive to osteogenic repair in load-bearing bone defects. Ideally, bone-grafting materials should satisfy the following requirements: osteoconductivity, osteoinductivity, biocompatibility, angiogenesis, mechanical properties matching natural bone, and controlled bioabsorption/biodegradation (Fig. 1) (de Grado et al., 2018).

3 Traditional bone-grafting materials for repairing CSDs in load-bearing bone

3.1 Autografts

Autologous bone transplantation is a process in which appropriate healthy bone tissue is harvested

Table 2 Mechanical properties of cortical bone and cancellous bone

Material	Compressive strength (MPa)	Flexural, tensile strength (MPa)	Strain to failure (%)	Fracture toughness (MPa·m$^{1/2}$)	Young's modulus (GPa)
Cortical bone	100–230	50–150	1–3	2–12	7–30
Cancellous bone	2–12	10–20	5–7		0.5–0.05

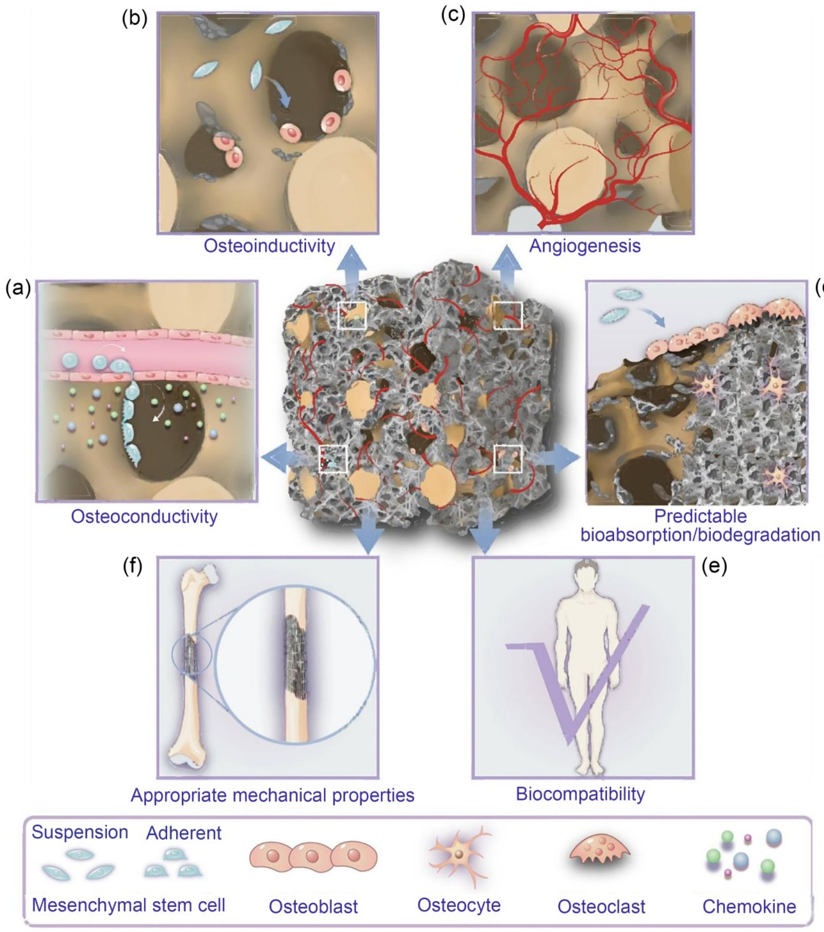

Fig. 1 Ideal properties of bone-grafting materials. (a) Osteoconductivity: simulating the three-dimensional (3D) porous structure of the natural bone ECM; providing enough space for cell growth; guiding cell migration, proliferation and differentiation, and vascular invasion; and finally forming mature bone tissue. (b) Osteoinductivity: inducing osteogenic differentiation of stem cells and new bone formation. (c) Angiogenesis: promoting the growth of new vessels into a bone graft. (d) Predictable bioabsorption/biodegradation: bone graft can be absorbed/degraded in the body at an appropriate rate without hindering the formation of new bone. Moreover, the rate of absorption/degradation should match the rate of new bone formation. (e) Biocompatibility: bone graft does not cause a serious immune reaction and is non-toxic and harmless in the human body. (f) Appropriate mechanical properties: simulating the shape and mechanical properties at the bone-defect site, and performing the mechanical support and motion functions.

from another site in the patient's own skeleton to implant at the bone-defect site. Autologous bone grafting is the current "gold standard" for repairing CSDs in load-bearing bone, and is commonly used as the therapeutic standard for allograft and artificial graft alternatives (de Long et al., 2007; Brydone et al., 2010). Autologous bone grafting has been widely used in clinical practice for decades, and the primary bone sources are the patient's own fibula, iliac bone, ribs, ulna, and radius (Haugen et al., 2019). At present, autologous bone grafts are generally used for weight-bearing bone defect repair in clinical settings (Figs. 2a–2c), including non-vascularized autologous bone grafts (such as iliac cancellous bone) and vascularized autologous bone grafts (such as free fibular flap, rib flap, and iliac flap with vascular pedicle) (Lin et al., 1999). Distraction osteogenesis, characterized by the gradual translocation of a segment of new bone from the healthy area into a region of bone loss, is also an effective method for treating load-bearing bone defects.

Autologous bone grafts have several advantageous properties that facilitate bone regeneration. Autologous bone tissue will not cause rejection reactions (Klijn et al., 2010), and living bone contains a large number of viable stem cells, osteogenic cytokines, and proteins that stimulate the migration, proliferation, and

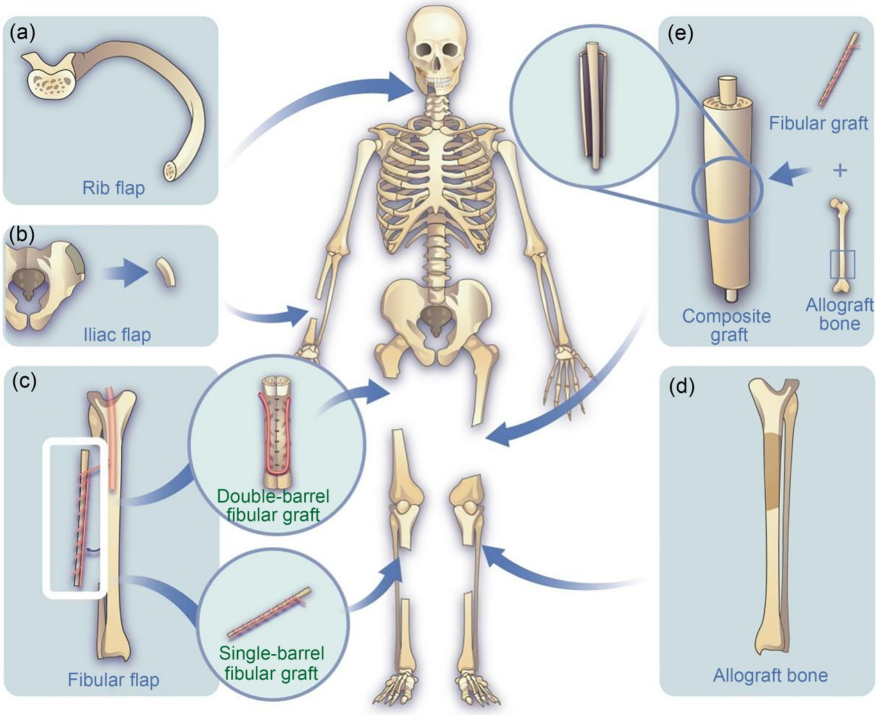

Fig. 2 Commonly used natural bone grafts. Autografts (bone grafts from the same individual) include rib flap (a), iliac flap (b), and fibular flap (c). Allografts (bone grafts from another individual) are commonly used to repair bone defects alone (d) or in combination with free vascularized fibular grafts (e).

differentiation of stem cells to form new bone (Dimitriou et al., 2011; Henkel et al., 2013). Bone ECM provides sufficient structural space for both vascular ingrowth and cell migration, growth, and osteogenesis. Therefore, autologous bone grafts have a significant effect on promoting bone regeneration in defect areas. A rich blood supply has a positive influence on the process and results of bone healing. Clinical studies have shown that non-vascularized autologous bone grafts are more prone to bone resorption and infection after implantation than vascularized autologous bone grafts. Through microvascular anastomosis, bone grafts with a vascular pedicle can supply the immediate demand for blood and provide a rich source of osteogenic growth factors and nutrients, which is beneficial for infection resistance and bone healing. Moreover, the healing process between the implanted bone tissue and surrounding bone tissue resembles the fracture-healing response, but with a faster healing rate and less bone resorption. This is conducive to early recovery of mechanical load-bearing and motor function as well as reducing the risk of pathological fractures. The bone defect can be repaired simultaneously with soft-tissue loss by means of a pedicled bone flap or free bone flap. Therefore, non-vascularized bone grafts are generally recommended for most small bone defects with adequate soft-tissue covering and a low risk of infection in the recipient area, while vascularized bone grafts are used mostly for large bone defects in the presence of a poor soft-tissue coverage, a high infection risk, and a chemo-radiotherapy requirement.

A free vascularized fibular graft (FVFG) is the most commonly used vascularized bone graft for repairing CSDs in load-bearing bone in clinical settings, and has produced satisfactory clinical outcomes (Kawate et al., 2007; Pederson and Person, 2007; Korompilias et al., 2011). With its characteristics of relatively dense structure, long and straight morphology, a large intercept range, and a predictable vascular pedicle, the fibular graft has certain advantages in the repair of load-bearing bone defects (Bach et al., 2004; Beris et al., 2011; Korompilias et al., 2011; Khira and Badawy, 2013; Ou et al., 2020). Furthermore, the anatomical structure of the fibula allows intramedullary dowelling of tibial, femoral, and humeral defects. A fibular graft can produce hypertrophic reactions due to microscopic stress fractures. This occurs in response to mechanical loading by protected weight bearing in most cases (Ceruso et al., 2008; Kovoor et al., 2011; Roddy et al., 2018). When a free fibular graft is used to repair a tibial or femoral defect, the hypertrophic response of the fibula enables it to provide the required load-bearing capacity for the defect site (Kovoor et al., 2011). Single-barrel fibular grafts are applied mainly in lower stress-loaded settings such as the upper limbs, the mid-tibia, and intercalary defects in children, while double-barrel fibular grafts are more suitable for large bone defect areas and moderate stress-loaded sites such as the femur (Bi et al., 2008; Zekry et al., 2019). Double bone mass is beneficial because it provides superior initial mechanical strength and biomechanical stabilization. The anatomical shape of the rib is thin and curved, which makes rib less effective than fibular for repairing large segmental defects in the limbs. Owing to the curved morphology of the iliac crest and its abundant cancellous bone tissue, an iliac flap pedicled with deep circumflex iliac vessels is more suitable for repairing defects in short bones with extensive soft-tissue loss.

However, there are several significant limitations in autogenous bone grafting (Sen and Miclau, 2007). Autografts are associated with a risk of donor-site morbidity due to surgical bone removal from another healthy site, which may lead to infection, bleeding, delayed healing, secondary deformity, and abnormal joint function at the donor site. Moreover, the relatively limited sources of autograft tissue make large defects problematic to repair. It is also difficult to obtain the desired bone graft shape for the defect site and achieve ideal functional reconstruction with this approach, especially for large bone defects with complex anatomical morphology and function. Merely restoring the continuity of damaged bone does not fully resolve the dysfunction caused by bone defects. In the early stage after implantation, overuse and repetitive loading increase the risk of stress fractures. In addition, the donor bone often requires a prolonged period of bone remodeling to achieve the strength to meet basic load-bearing demands.

3.2 Allografts

In allografts, the donor bone tissue is extracted from another individual of the same species and transplanted into the patient's bone defect. Allografts can be used as an alternative to autografts because they offer comparable structural and mechanical properties, provide the same osteoconductive conduits to induce bone regeneration, and provide sufficient load-bearing support. Allogeneic bone is convenient to harvest, is relatively easy to source, and causes less damage to patients. Allografts are reported to be effective in repairing load-bearing bone defects (Figs. 2d and 2e). Nevertheless, they are not extensively used in clinical application, owing mainly to the potential risks of disease transmission, immune rejection, or limited osteoinductivity (Kolk et al., 2012).

To minimize immunogenicity and extend preservation time, allogeneic bone should be physically or chemically processed (for example by deep cryogenic freezing, freeze-drying, chemical treatment, irradiation, or decalcification) to maintain its physical structure and osteoconductive properties. However, compared to living bone, processed allograft bone loses cells and osteoinductive cytokine activity, which leads to a lack of osteogenic and osteoinductive potential (Habibovic and de Groot, 2007; Szostakowski and DeMaio, 2020). Thus, allografts act mainly as osteoconductive scaffolds. Allograft healing is primarily achieved by the process of "creeping substitution," which involves the host bone penetrating allograft bone channels and gradually resorbing the allografts, as well as formation of new bone (Ogilvie et al., 2009). A stable and firm internal fixation is conducive to accelerate incorporation of allografts into host bone, as well as early functional recovery. Transplantation of allogeneic bone combined with autogenous cancellous bone can effectively promote osseointegration. Furthermore, binding of active osteoinductive cytokines and cells to allografts can enhance bone integration to improve the bone-healing effect (Zhang et al., 2020).

CSDs in weight-bearing areas are difficult to repair with allogeneic bone grafts because of the high dependence of these grafts on the blood supply of the recipient area, the long process of revascularization, slow healing, and infection. Capanna et al. (2007) proposed combining allografts with FVFGs to repair large load-bearing bone defects caused by tumor resection. FVFG can provide a rich blood supply and osteogenic capacity, promoting good integration between the allograft and the surrounding host bone and reducing infection and failure rates (Innocenti et al., 2009). Allogeneic bone provides adequate mechanical support for a fibular graft, allowing the recipient area to gain an early load-bearing capacity. The stimulation of load-bearing stress contributes to progressive hypertrophy of the fibular graft, which compensates for the weakened mechanical properties of the allografts due to bone resorption (Rabitsch et al., 2013; Zekry et al., 2019). In a study performed by Venkatramani et al. (2015), six patients with distal femoral bone defects (with an average defect length of 15 cm) received reconstructions with allografts combined with FVFGs. All cases healed well, with an average healing time of six months. Similar results were also demonstrated by other researchers. Halim et al. (2015) suggested that free fibular grafts with a vascular pedicle, combined with allografts, could increase initial stability and yield good results, and are beneficial for achieving early weight bearing and good bone healing. Ozaki et al. (1997) improved the Capanna technique using pedicled fibular grafts from the same side of the defect, combined with allografts, to reconstruct the tibia. A retrospective study by Manfrini et al. (2017) claimed that allografts supplemented with pedicled fibular grafts had obvious advantages over free vascularized fibular grafts. Compared with FVFGs obtained from the opposite side, pedicled fibular grafts obtained from the ipsilateral side of defects had similar prognostic outcomes, a convenient surgical process, shorter operation time, and fewer complications. Fascial flap-wrapped allografts are also a promising development as a viable method for repairing large weight-bearing bone defects. Several studies have established that fascial flap-wrapped allogeneic bone is feasible for initial revascularization of allografts and is superior to allogeneic bone alone for bone defect repair (Giessler et al., 2009; Dou et al., 2016).

4 Novel bone grafts: from "bone tissue engineering scaffolds" to "bone-grafting substitutes"

Owing to the limitations of natural bone grafts, research on obtaining novel bone grafts that can both offer similar therapeutic effects and overcome these limitations has begun in recent years (de Grado et al., 2018). Novel bone grafts refer to synthetic biological materials that can be used to repair bone defects and promote bone healing, as an alternative to traditional bone grafts. With the rise of tissue-engineering technology, the application of tissue-engineered bone has become a hot topic in the area of CSD reconstruction. A variety of biomaterials have been found that can be used as tissue scaffolds for bone tissue engineering. Tissue scaffolds are the core and foundation of bone-tissue engineering, and are combined with seed cells and/or cytokines to recreate native bone tissue (Cao and Kuboyama, 2010; Zhang B et al., 2017; Xu et al., 2019; Su et al., 2021). Tissue scaffolds provide space for cell growth and proliferation, and cytokines induce differentiation of stem cells into osteoblasts, as well as vascularization and subsequent bone formation (Cao and Kuboyama, 2010). Ideally, biological scaffolds should allow the normal formation of new bone, and their degradation rate should correspond with the degree of new bone formation. The newly formed bone continuously replaces the degraded biological materials, and eventually, the defect is completely repaired by the newly formed bone (Su et al., 2021). Compared with traditional bone grafts, these alternatives have largely solved the problem of bone deficiency and prevented cross-infection and immune rejection. In addition, novel bone grafts can be designed with the desired shape and mechanical properties according to the requirements of the bone defect site.

Because most scaffolds for bone tissue engineering cannot provide sufficient mechanical strength, in recent years, research focused in novel bone grafts for load-bearing bone defects has gradually returned to bone-grafting substitutes. In the design and application of novel bone grafts, it is important to consider what effect the biomaterials transplanted into the recipient area should achieve. The term "tissue-engineering scaffold material" emphasizes acting as a scaffold to provide a three-dimensional (3D) environment for migration, proliferation, differentiation, and osteogenesis of seed cells, while "bone-grafting substitute" emphasizes replacing the normal function of bone tissue at defect sites. The focus of bone defect repair is not only preservation of the limb and aesthetic appearance, but also restoration of the original load-bearing capacity and motor function, to the extent possible (Dong et al., 2017). This means that the bone graft needs to have sufficient initial mechanical strength during the early stage of osteogenesis to maintain load-bearing capacity (Khan et al., 2021). In addition, the implant should maintain mechanical stability and structural integrity when the newly formed bone is immature; otherwise, bone regeneration will fail. However, the most bioabsorbable or biodegradable biological scaffolds cannot provide the mechanical strength required for load-bearing sites, even with the aid of external fixation. Poor mechanical strength is the main cause of failure after implantation in load-bearing areas, and implants are prone to deformation, fracture, and loosening under stress (Zhong et al., 2019). However, it is unknown whether the future research direction of novel bone grafts will return to "bone-grafting substitution" (emphasizing functional restoration) or move into new development areas.

Tissue-engineered bone requires strict laboratory conditions and cumbersome maintenance of cell cultures, and is costly, hindering its widespread clinical application. A series of problems in tissue engineering, such as maintaining activity of cells and growth factors, as well as steady-state active factor concentration, and the in vivo stability of bioactive-substance delivery systems, need further study. Some researchers have suggested that the development of tissue-inducing biomaterials without the need for exogenous cells or additional bioactive molecules is the frontier of development (Yuan et al., 2022). Tissue-inducing biomaterials have the intrinsic biological ability to induce tissue regeneration progressively without the addition of cells and/or bioactive factors (Yuan et al., 2022). Development of novel bone grafts with highly efficient osteoinductive activity for bone regeneration is therefore of great significance.

Thus, novel bone grafts for bone defect repair should have specific features (including good osteoinductivity, osteoconductivity, angiogenesis, and mechanical properties matching natural bone) in order to be considered as ideal materials for grafting at load-bearing sites.

4.1 Development strategy for bone grafts based on substance bionics

Selection of appropriate bone-grafting substitutes is crucial for adequate bone formation at defect sites. Clinical demand for excellent bone-grafting substitutes is motivating the development of novel bone biomaterials. A variety of substitutes have potential for replacing natural bone grafts, but it is hard to find a single material that offers all the desired properties required for bone defect repair in load-bearing bones. Development of bone-grafting substitutes should be based on the advantages of various types of biomaterials and pursue promising solutions to overcome their inherent disadvantages. Combining the advantages and maximizing the performance of different bone grafts through synthesis of composite materials has been an effective strategy for improving the physicochemical and biological properties of substitutes.

4.1.1 Metallic bone-grafting materials

Owing to its high mechanical strength and good fatigue resistance, medical metal is widely used in the clinical treatment of load-bearing sites such as artificial bone prostheses, artificial joints, and implant fixation devices. Non-degradable medical metal materials (such as titanium and titanium alloy, tantalum, stainless steel, and cobalt-chromium alloy) generally have some excellent properties including good mechanical properties, stable physicochemical properties, good processability, anticorrosion performance, and biocompatibility. Therefore, metals have been extensively used in the repair of load-bearing bone defects over recent decades, and are commonly applicable as long-term or permanent load-bearing implants.

The differences between the mechanical properties of metal materials and natural bone tissue are one of the important reasons for their limited clinical application. The elastic modulus of most metal materials is significantly higher than that of natural bone, which results in the "stress shielding" effect (Long and Rack, 1998; Noyama et al., 2013; Maradze et al., 2018; Kunii et al., 2019). This effect causes insufficient physiological load transfer from metal implants to adjacent bone and subsequent bone absorption because of the reduced stress stimulation. This is not conducive to the long-term stability of metallic grafts in vivo (Noyama et al., 2013; Kim et al., 2020). In addition, the harmful biological responses caused by release of metal ions or shedding of metal particles during long-term implantation also deserve attention. The interface between bioinert metallic materials and surrounding bone tends to form fibrous connective tissue rather than a strong bond with bone. The bio-inertness of metals contributes to poor osteogenesis and inhibits osteointegration between the material and bone, adversely affecting initial stability and long-term application in load-bearing areas.

The traditional view is that free cancellous bone grafts are not suitable for the repair of large segmental bone defects because of their looseness and frequent bone resorption. A modified method called "wrapped cancellous bone graft" overcomes this shortcoming using cancellous bone wrapped in a mechanical device, which makes it one of the most effective methods of repairing large-segment bone defects, particularly in load-bearing bone (Ayvaz et al., 2018; Ma et al., 2019). Cobos et al. (2000) first reported the successful use of titanium mesh-wrapped cancellous bone grafts to repair two large-segment tibial bone defects in different patients. On the basis of reduction and fixation of the bone defect sites, titanium mesh is used as an external scaffold, and is filled with autogenous cancellous bone, cortical bone, allogeneic bone, or artificial bone materials. The titanium mesh tightens the bone-grafting material and firmly fixes it at the proximal and distal ends of the bone defect (Ma et al., 2019). The titanium mesh mechanically wraps and fixes the internal grafting material, closely connecting it to the surrounding bone and the internal fixation devices, achieving excellent stability (Attias and Lindsey, 2006; Bullens et al., 2009b). In addition, the titanium mesh prevents (to a certain extent) undesirable stimulation from external muscle activity and the growth of fibrous tissue, thus providing a good environment for internal osteogenesis (Lindsey et al., 2006). The pore structure of the titanium mesh allows the internal grafting material to obtain oxygen and nutrients from the surrounding tissue without obstructing the ingrowth of blood vessels or callus formation. One study compared the efficacy of titanium mesh encasing cancellous bone and large cortical bone grafting in the treatment of large-segment bone defects in goat femurs (Bullens et al., 2009a). After six months, the defect site in the titanium mesh group was completely replaced by new bone and the torsional strength reached 66.6% of that of the contralateral femur. The bone-healing effect and mechanical properties of the titanium mesh group were significantly better than those of the large cortical bone group (Ma et al., 2019). Compared with traditional cancellous bone grafts, the wrapped bone graft method requires a larger amount of cancellous bone to be implanted at the defect site, and the amount of bone graft required is approximately 1.5–2.0 times that of the bone defect. This provides more active cells and cytokines to facilitate bone formation and leads to a relatively low rate of bone absorption (Ma et al., 2019). If the amount of autogenous cancellous bone is insufficient, no more than 1/4 cortical bone or artificial bone can be added. However, it is difficult to remove the titanium mesh after bone healing, so it is necessary to consider the stress shielding and metal toxicity caused by long-term implantation of titanium mesh. The question of how to restore the normal structure of the cortex and medullary cavity after implantation also requires further research.

To avoid potential problems caused by long-term implantation of non-degradable metals, a new trend has emerged to develop degradable metal materials with excellent mechanical properties that can meet load-bearing requirements. At the initial stage of implantation, biodegradable metal materials can achieve loading and stability with high mechanical strength. During the middle stage of implantation, the metal gradually degrades, which reduces its hardness and provides mechanical stimulation for bone formation. Ultimately, the metal is completely degraded and replaced by new bone, preventing the damage and difficulty of a second surgery to remove the metal (Ding et al., 2014). Degradable magnesium metal grafts have received increasing attention from front-line researchers for clinical application in bone defect repair, and there are already commercial bone implant products (Ding et al., 2014; Azadani et al., 2022). In addition to their excellent biocompatibility and biodegradability, magnesium metal materials also have mechanical properties more similar to those of natural bone. Compared to traditional metal materials such as titanium alloy, magnesium has an elastic modulus of 41–45 GPa, quite close to that of natural bone (Hu et al., 2021; Prithivirajan et al., 2021). Magnesium ions released by the corrosion of magnesium materials are indispensable trace elements in the human body and significantly alter the cells' metabolic and proliferative activities, further influencing cell differentiation (Maradze et al., 2018). Mg concentrations below 10 mmol/L are beneficial for cell growth, whereas high Mg concentrations have an adverse effect on osteoclasts (Maradze et al., 2018). It is necessary to find a way to accurately control the degradation rate of biodegradable metal materials before proposing them for clinical application. If the rate of degradation is much faster than that of bone formation, the stiffness of the bone-metal composite will be insufficient to support mechanical stress, thus potentially resulting in graft loosening and pathological fracture. Owing to the rapid corrosion of pure magnesium, it is difficult to maintain good mechanical stability during bone healing (Prithivirajan et al., 2021). Moreover, rapid dissolution can lead to increased concentrations of hydrogen and magnesium ions in the local environment, which is not conducive to bone healing (Hu et al., 2021). Therefore, researchers often use alloying to improve the match between the rate of magnesium degradation and osteogenesis. Reducing the content of impurities (Fe, Ni, Cu, and Co) in magnesium materials and developing new magnesium alloys by adding different proportions of elements such as Ca, Zn, Sr, Mn, Zr, and rare earth elements are improvement strategies commonly used in current research (Song, 2007; Ding et al., 2015; Chen et al., 2020; Prithivirajan et al., 2021). The addition of silver to magnesium metal materials not only improves their mechanical properties and corrosion resistance, but also improves their ability to repel infection, due to the antibacterial activity of silver.

4.1.2 Bioceramic bone-grafting materials

Bioceramics have attracted wide attention because of their excellent biocompatibility, relatively stable physicochemical properties, good compression resistance and wear resistance, and outstanding bone induction ability. The chemical composition and microstructure of bioceramics simulate the mineral composition of natural bone and its 3D porous structure, so bioceramics can provide an osteoconductive interface. Bioceramic materials commonly used in bone defect repair are mainly bioactive ceramics such as calcium phosphate bioceramics (including hydroxyapatite (HA), β-tricalcium phosphate (β-TCP), and their composites), which are similar in mineralized composition to natural bone tissue, and bioactive glass composed of oxides of silicon, calcium, phosphorus, and sodium (Gerhardt and Boccaccini, 2010; Feng et al., 2012; Ganesh et al., 2014; Patel et al., 2020).

Calcium phosphate ceramics are considered one of the most promising types of biomaterials for future clinical application because of their excellent bone conduction and bone induction ability (Jeong et al., 2019; Xiao et al., 2020). Biphasic calcium phosphate (BCP) is composed of β-TCP with a high dissolution rate and HA with a low dissolution rate in an appropriate ratio, which combines the advantages of both good solubility and stability, and thus has a suitable degradation rate (Kim et al., 2019). The osteoinduction of calcium phosphate ceramics is based on dissolved calcium and phosphorus ions, a porous structure, and surface adsorption of active proteins. Calcium and phosphorus ions play an important role in osteogenesis induction. Ca^{2+} is an important homing signal that stimulates the migration, proliferation, and differentiation of osteoblast-related cells (Guo et al., 2020), and PO_4^{3-} plays a key role in the mineralization of physiological bone matrix (Murshed et al., 2005). In addition, the affinity of calcium and phosphorus to proteins leads to adsorption of osteogenic proteins.

However, the inherent brittleness of calcium phosphate bioceramics limits their application in load-bearing areas, and they are more often used to fill small or non-segmental bone defects in the form of scaffolds, particles, or bone cement (Xiao et al., 2020). In recent years, the potential of silicate bioceramics as bone repair materials has gradually emerged (Yu et al., 2013). Calcium silicate ceramics also exhibit good biological activity, and the released calcium and silicate ions play a role in the process of bone formation (Zhang WJ et al., 2017). The silicon and calcium ions released by calcium silicate bioceramics can stimulate human umbilical-vein endothelial cells and hemoglobin MSCs to produce growth factors, bone morphogenetic proteins, and related enzymes, thereby promoting angiogenesis and osteogenic differentiation (Li et al., 2014). Moreover, most calcium silicate ceramics have an elastic modulus and bending strength similar to those of human bone cortex, unlike traditional calcium phosphate ceramics.

Doping bioceramics with Sr, Zn, Mg, and Zr is expected to produce novel bioceramics with better mechanical and biological properties (No et al., 2017). Tarafder et al. (2013) compared the effects of pure TCP scaffolds with composite TCP scaffolds (doped with MgO and SrO) in rat distal femoral defect models. They found that TCP scaffolds doped with MgO and SrO significantly increased the formation of osteoid-like new bone. They also observed an acceleration of internal mineralization as well as an increase in osteocalcin and type I collagen in serum in the composite TCP group. In another study, researchers designed bioceramic composites by combining HA with ZrO_2 to endow HA with high mechanical strength. When the ZrO_2 content increased from 50% to 100% (mass fraction), the compressive strength of the scaffold increased from 2.5 to 13.8 MPa. Moreover, compared with the pure ZrO_2 scaffold, ZrO_2/HA scaffold showed better affinity for cellular adhesion and proliferation (An et al., 2012).

4.1.3 Polymer bone-grafting materials

Depending on the source of raw materials, polymers used as bone-grafting materials can usually be divided into either natural or synthetic polymers (Guo and Ma, 2014). Natural polymers were some of the earliest biomaterials used in clinical settings. Generally, they show appropriate biological properties such as excellent cell affinity, biocompatibility, and degradability, and negligible toxicity to degradation products, making them suitable for biomedical application. Representative natural polymer bone graft materials include proteins such as collagen (Wahyuningtyas et al., 2019), gelatin (Hamlekhan et al., 2011), and fibrin (Kohli et al., 2021), and polysaccharides such as hyaluronic acid (Zhai et al., 2020), alginate (Kohli et al., 2021), dextran (Nikpour et al., 2018), and chitosan (Marín et al., 2019). However, natural polymers have some drawbacks, such as low mechanical strength, an unpredictable degradation rate, unstable sources, high solubility, denaturation during processing, and potential immunogenicity or pathogenic impurities (Salgado et al., 2004). In particular, the lack of mechanical strength greatly limits their usefulness for repair of bone defects in load-bearing areas. However, natural polymers with good cellular activity can be combined with other biomaterials and thus improve the properties of grafts to meet the requirements of repair in load-bearing areas. The most appropriate inspiration for bone-grafting material is natural bone tissue itself. Natural bone tissue is composed of organic matter such as collagen and inorganic matter such as HA. Therefore, it should be feasible to develop bionic materials composed of organic materials and inorganic bioceramics or polymers (Antebi et al., 2013; Xie et al., 2019). The design of collagen/HA composites that closely mimic natural bone chemistry and microstructure leads to better bone repair (Lee et al., 2017). One group of researchers deposited multilayer silk fibroin on a BCP scaffold to reduce its brittleness and increase its mechanical strength (Li et al., 2013). The results showed that the mechanical properties of BCP scaffolding could be improved by increasing the amount of silk fibroin coating, and the compressive strength of BCP could be increased by six times with five layers of coating. In addition, the silk fibroin/BCP material played a positive role in long-term osteogenesis of MSCs.

Synthetic polymers with high design flexibility can be tailored with controllable mechanical properties, microstructure, and degradation rates by adjusting synthetic parameters, and are suitable for large-scale manufacturing. At present, the main synthetic polymers used in bone regeneration and repair include polylactic acid (PLA) (Unagolla and Jayasuriya, 2019), polyglycolic acid (PGA) (Ko et al., 2021), polycaprolactone (PCL) (Hamlekhan et al., 2011), and polyaryletherketone (PAEK) (Feng et al., 2020; Yuan et al., 2022). Synthetic polymers generally have better mechanical strength than bioceramics, but have poor cell adhesion and weak biological activity. Some synthetic polymers produce acid degradation products during degradation in vivo. The reduced local pH value not only has an adverse effect on the activity of surrounding cells and causes inflammation, but also may increase the graft degradation rate (Garric et al., 2017). Meanwhile, acid products may unfavorably affect the activity of acid-sensitive drugs when used as drug carriers. Most synthetic polymers exhibit weak cell attachment because of their hydrophobicity and lack of surface-cell recognition sites (Guo and Ma, 2014; Dong et al., 2017).

The mechanical properties of most degradable polymer materials are not adequate to support the stress in load-bearing bones. Non-degradable polymer materials with excellent mechanical properties are still highly valued in the search for suitable materials for this purpose. PAEK is a kind of non-degradable synthetic polymer with excellent mechanical properties, and has been widely used in orthopedics, spine repair, and other medical fields. The most typical PAEKs are polyetheretherketone (PEEK) and polyetherketoneketone (PEKK) (Zhong et al., 2019; Yuan et al., 2022). PAEK has good biocompatibility, corrosion resistance, thermal stability, and mechanical properties similar to those of natural bone, which means that it could be used as a long-term or even permanent implant. Compared with titanium alloy, the elastic modulus of PAEK is closer to that of bone tissue (Zhong et al., 2019). To better simulate the mechanical and biological properties of bone tissue, ceramics, titanium, carbon nanotubes, carbon fibers, and other materials are commonly used to fill or modify PAEK. However, bioinert PAEK materials cannot easily achieve good integration with the host bone, which greatly restricts their clinical application in load-bearing bone defects. One effective approach is to prepare bioactive PAEK composites by blending bioactive materials into PAEK substrates (Han et al., 2019).

4.2 Development strategy for bone grafts based on structural bionics

Bone tissue is a structural composite with a highly ordered arrangement of organic and inorganic compounds, exhibiting unique micro and macro structural characteristics (Tian et al., 2019). As artificial bone substitutions, bone graft materials often benefit from mimicking advantageous structural features of the natural bone ECM. In addition to designing organic-inorganic composite materials such as collagen-bioceramics to simulate the composition characteristics of bone tissue (Ji et al., 2022), simulating the 3D architecture of bone ECM is an important strategy for improving the osteogenic properties of bone-grafting materials (Marí-Buyé et al., 2013).

4.2.1 Pore architecture

Pore architecture, such as pore size, porosity, and connectivity, greatly influences cell behavior, in-growth of vessels, and bone regeneration. The bioinspired porous structure of biomaterials can provide essential space and guidance for migration of surrounding cells and the growth of blood vessels (Li et al., 2006). Several studies suggest that the pore size of bone repair materials should be at least 100 μm to ensure the delivery of nutrients and oxygen (Karageorgiou and Kaplan, 2005; Carpenter et al., 2016). Pore size within a biomaterial will affect the type of tissue regenerated at the defect site. Large pores (100–150 μm and 150–200 μm) result in a large amount of mineralized bone tissue growing inward, while medium pores (75–100 μm) result in no mineralized osteoid tissue growing inward and small pores (10–44 μm and 44–75 μm) cause only fibrous tissue growing inward (Hulbert et al., 1970; Rouwkema et al., 2008). Micropores (<10 μm) also have a positive effect on new bone formation (Levengood et al., 2010; Hayashi et al., 2019). It has been proved that a high degree of microporosity is crucial for osteoconduction and creeping substitution (Ghayor et al., 2020). Micropores can provide a relatively large specific surface area, which reflects the amount of space for interaction between the biomaterial and surrounding tissues. A larger surface area contributes to bone-induced protein adsorption, cell adhesion, biomaterial degradation, and apatite crystal deposition (Karageorgiou and Kaplan, 2005). The new bone tissue grows into micropores to form mechanical chimeras, which produce better mechanical stability. Some studies have shown that scaffolds with multiple pore sizes have better repair results than materials with only large pores (Cooper et al., 2004; Woodard et al., 2007). Therefore, a gradient porous structure may be more appropriate for the design of bone-grafting materials. Connectivity between pores is also important in bone regeneration, allowing the exchange of substances, cell adhesion, and ingrowth of surrounding tissue. At the same time, the size of pore intercommunication channels also affects the degradation rate and mechanical properties of materials. Channel sizes in the 15–50 μm range are generally recommended as beneficial to the flow of cells and nutrients. Shibahara et al. (2022) designed three kinds of carbonate apatite HC scaffolds with different volume proportions of channels and micropores. After 12 weeks, the HC scaffold with a large number of channels and micropores demonstrated an increased amount of bone formation. The results showed that scaffold channels influenced early bone growth, and micropores influenced scaffold absorption and bone formation during the middle stage. The influence of pore structure on the mechanical properties of bone grafts should also be considered. There appears to be an inverse relationship between compressive strength and porosity/pore size (Miri et al., 2024). The challenge is to develop biomaterials that can both meet the mechanical requirements to withstand static and dynamic loads and maintain sufficient pore structure to facilitate cell ingrowth and vascularization.

4.2.2 Irregular porous structure

Current studies have shown that bone-grafting materials with an irregular porous structure are likely to have better bionic properties, because this structure is conducive to osteoblast activity (Zhu et al., 2021). Natural bone is characterized by irregular pore size and shape, trabecular diameter, and hierarchical structure, resulting in heterogeneous and anisotropic mechanical properties. The design of most porous materials, on the other hand, is based on the repetition of regular pore structures in 3D space (Chen et al., 2021). Despite the advantages of a simple structure, manageable pore parameters, and predictable mechanical properties, bone scaffolds designed with regular porous structure cannot accurately simulate the morphological characteristics of natural bone. Irregular porous scaffolds can simulate the complex anisotropic structure of bone tissue, so as to achieve excellent biomechanical bionic properties. Some researchers have combined a load adaptation algorithm with a mechanobiology algorithm to design an irregular load-adapted scaffold that is able to withstand complex load distribution (Rodríguez-Montaño et al., 2019). Under three different sets of boundary and loading conditions, the performance of the irregular load-adapted scaffolds was better than that of regular scaffolds. It has been found from computed tomography (CT) images that natural bone can be expressed as a Voronoi tessellation, which is a method of dividing areas into units based on randomly scattered areas or seed points (Zhao et al., 2022). Wang et al. (2018) used the top-down design method based on Voronoi tessellation to build an irregular porous scaffold with hierarchical porosity, achieving good control of the irregularity of the porous structure. Moreover, mechanical experiments showed that this irregular scaffold exhibited an elastic modulus and compressive strength similar to those of natural bone. Zhao et al. (2021) also designed a gradient irregular porous scaffold based on Voronoi tessellation to simulate the morphologic characteristics and elastic modulus of bone. Through finite element analysis, they found that the graded irregular porous structure had better stability and impact resistance, which was favorable for maintaining stability and bearing the impact force conducted by the surrounding bone. Furthermore, the random structure of scaffolds based on Voronoi tessellation led to irregular permeability similar to that of natural bone, guaranteeing transport of nutrients and oxygen (Zhao et al., 2022). However, at present, the optimum pore size, porosity, pore structure, or other microscopic parameters of bone graft materials suitable for bone regeneration in load-bearing areas have not been determined.

4.2.3 Fabrication of bone grafts with bionic structure

The fabrication of bone grafts with a bionic structure is inseparable from the precise control of pore shape, size, and distribution, penetration rate, surface structure, and other parameters. The appearance of 3D printing techniques provided the technical basis for the manufacture of biomimetic structural materials (Ma et al., 2018). Three-dimensional printing is a kind of additive manufacturing technology characterized by digitalization, personalization, and customization, including melt deposition molding, selective laser sintering, light-curing molding, and electron-beam melting. It is used to optimize osteogenic properties and achieve personalized design and production of synthetic bone-grafting materials. Traditional manufacturing technologies for porous structural materials (such as particle leaching, freeze-drying, and gas foaming) provide little to no control for precise modification of structural characteristics of bone-grafting materials, making it difficult to optimize these properties accurately and predictably (Ma et al., 2018; Shi et al., 2018). Therefore, biomaterials prepared by 3D printing technology have unique advantages in accuracy, personalization, parameter regulation, spatial structure complexity, and other aspects. In terms of macrostructure, 3D printing can replicate complex anatomical shapes based on patient-specific data from bone defect sites. In terms of microstructure, 3D printing can construct bone-grafting materials with internal morphology mimetics and biomechanical properties similar to those of natural bone (Ma et al., 2018). In a study of 26 patients, researchers used 3D printing technology to design personalized porous titanium grafts (pore size of 400–600 μm, strut diameter of 240–320 μm, and porosity of 60%–80%) for reconstruction of large bone defects without autogenous or allogeneic bone (Zhang et al., 2021). The results showed that all patients achieved good bone healing, and the 3D-printed porous titanium grafts led to immediate and long-term biomechanical stability after surgery. Results from sheep models of a femur defect also showed that new bone grew into the pores and gradual remodeling affected bone integration, resulting in long-term mechanical stability of the implant. However, a large number of design variables related to bone regeneration limit the predictability of the clinical bone-healing efficacy of bone graft materials, which means that repeated trials and exploration are required.

4.3 Development strategy for bone grafts based on functional bionics

There are two main biointegration mechanisms that occur in bone-graft implantation, either indirectly through the fibrous tissue layer on the implant surface (fiber–bone integration) or directly through the formation of new bone on the implant surface (osseous integration) (Divakarla et al., 2018). Bioactive grafts tend to form a tight bone bond, while bioinert bone grafts such as PAEK and titanium alloy merely repair the physical shape of bone defects, and commonly lack the ability to induce bone regeneration and form good bone integration. High-quality bone integration at the implant–bone interface is one of the key factors affecting the long-term survival of implants. To improve biocompatibility and bioactivity, several promising strategies have been studied and verified, including the construction of a porous structure, physical or chemical surface modification, and incorporation of bioactive materials into bioinert materials (Almasi et al., 2016; Dou et al., 2018; Zhong et al., 2019; Wan et al., 2020).

4.3.1 Surface properties of bone grafts

After implantation of exogenous grafts in vivo, the initial reaction is recognition and interaction between the implant surface and surrounding tissue (von Erlach et al., 2018). The surface properties of bone grafts (such as surface topography, roughness, and chemical composition) affect the adsorption of materials and cell behavior at the implant–bone interface, both of which play pivotal roles in biocompatibility and osseointegration (Boyan et al., 1996). Many studies have demonstrated that surface modifications such as plasma treatment, sandblasting, acid etching, and oxidation treatment can change the surface morphology of materials or allow the introduction of complex active moieties onto the surface, thus substantially altering the surface properties of grafting materials (Saruta et al., 2019). A porous and rough surface structure can increase mechanical interlocking between a biomaterial and adjacent bone tissue, thus providing stronger mechanical stability at the implant–bone interface. Surface modification to increase surface hydrophilicity of bone-grafting materials is also beneficial for the interaction between cells and biomaterials and promotes bone regeneration. The osteoblast adhesion ratio is higher in materials with a rough porous surface (Bowers et al., 1992), and the volume of bone formation in bone graft materials with high surface roughness is significantly larger than that in materials with low surface roughness (Zhang et al., 2015). The local cell–substrate attachment powerfully determines cell shape and function. When the cell membrane is stimulated by the surface morphology of the material, specific signals are transmitted through the cytoskeleton to the nucleus, thereby resulting in the expression of specific proteins and defining the phenotype of cells and tissues (Henkel et al., 2013). The high specific surface-area ratio provided by the surface micro/nano structure of biomaterials can enhance the attachment, proliferation, and differentiation of anchor-dependent cells associated with osteogenesis (Karageorgiou and Kaplan, 2005). Osteoblasts attach indirectly to the surface of biomaterials by attaching to the ECM protein adsorbed on the material's surface. Nanoscale surface roughness increases adsorption of ECM proteins, thus promoting the attachment of osteoblasts (Kim et al., 2021). It has been demonstrated that nano-/micron-scale surface features promote osteogenic differentiation of MSCs compared to smooth surfaces (Faia-Torres et al., 2014). Therefore, suitable surface properties are a vital consideration when assessing bone-grafting materials because of their influence on cell attachment, differentiation, and bone formation.

Among the options for restoring CSDs in load-bearing areas, one promising strategy is forming a bioactive material coating on the surface of bioinert materials with high mechanical strength, which does not adversely affect their intrinsic mechanical properties. Coating materials can be deposited onto the substrate surface by many techniques such as spraying, solution deposition, and plasma electrolytic oxidation (Momesso et al., 2020). In addition to the biological activity of the coating, the binding strength, uniformity, strength of the coating itself, and maintenance of biological activity also influence the effectiveness of coating materials in practical application. Among bioinert materials such as titanium alloy and PEEK, the most studied bioactive coating materials are apatite materials such as HA, which has well-recognized bioactivity and bone-inducing effects (Kawasaki et al., 2020). A nanoscale HA coating offers a bionic composition and bone–ECM structure on the surface of bone-grafting materials. Yuan et al. (2022) successfully developed bone-induced porous PEKK materials by depositing a nanoscale apatite surface coating, which significantly improved bioactivity, bone induction, and osseous integration. The apatite-modified porous PEKK not only induced ectopic osteogenesis in the dorsal muscle implantation site, but also led to greater bone healing in the distal femur defect model compared to uncoated PEKK. Titanium dioxide, magnesium, collagen, growth factors, and other materials can be used as coating and filling materials as well to enhance bio-performance (Almasi et al., 2016).

4.3.2 Mechanical loading and mechanical stimulation transmitting of bone grafts

Bone is a mechanically sensitive tissue. Physiological levels of mechanical stimulation are required to maintain osteocyte viability (Kogawa et al., 2018). Bone cells can sense and respond to external mechanical signals and translate physical stimuli into biochemical signals by mechanotransduction to regulate cell behavior (Toworfe et al., 2010; Mui et al., 2016; Ma et al., 2023). Implants used in load-bearing areas should ideally have good mechanical properties. Some scholars have argued that bone grafts need not provide exactly the same mechanical properties as healthy bone, but should at least be stiff and strong enough to provide structural support and transfer regeneration-enhancing forces to the host tissue (Reichert et al., 2011). Stress shielding is one of the key problems that affect the healing success of bone-grafting materials with good mechanical properties, such as titanium. The source of the problem lies in the apparent difference of elastic modulus between grafting materials and bone, which results in difficulty in transmitting stress from the implants to the host bone. The lack of mechanical stimuli increases osteocyte death and bone resorption, leading to loosening or even fracture of the implants. The elastic modulus of materials can be adjusted much closer to that of the skeleton by using a porous architecture with proper porosity, pore size, and other parameters (Hutmacher et al., 2007). However, increased porosity and pore size lead to reduced mechanical properties and diminish the structural stability of bone-grafting materials. To meet the needs of load-bearing bone defect repair, it is necessary to seek a balance between avoiding stress shielding and maintaining long-term stability. Owing to changes in age and health status, there are obvious differences in the elastic modulus of bone in different individuals. Therefore, it is necessary to customize and personalize bone implants for specific recipients.

Because proper mechanical stimulation has positive effects on bone regeneration, some scientists have attempted to optimize the structural design of bone-grafting materials to provide the best initial mechanical stimulation for bone regeneration in defect areas (Adachi et al., 2006; Chen et al., 2011). However, this optimal design provides the best mechanical stimulation only immediately after implantation, which is only one time point of the healing process. Bone regeneration is a highly dynamic process and involves the formation and resorption of distinct tissue types at specific locations in different stages, leading to changes in the mechanical environment over time. Owing to the continuously changing microenvironment, the mechanical stimulation delivered by the bone-grafting material may be affected by the new tissue. Thus, the best initial mechanical stimulation provided by materials may not be maintained throughout bone healing. Bashkuev et al. (2015) argued that it was not appropriate to have optimal initial mechanical stimulation as the objective of optimization. They proposed that the ideal mechanical stimulation should be provided for bone formation at every time point during the entire process of bone healing. Using finite element models for parameter optimization, they optimized the material properties (Young's modulus) in a given scaffold design and allowed the scaffold properties to change (the Young's modulus gradually decreased) during the bone-healing simulation, thereby providing the best mechanical stimulus for dynamic bone healing. Wu et al. (2021) also proposed a topologically optimized scaffold based on time-dependent mechanobiology, aiming to improve the long-term outcome of bone growth within the scaffolds. They first developed a topology-optimization algorithm based on the level-set method, and a time-based shape derivative to optimize the scaffold structure. They demonstrated its effectiveness by simulating a large bone defect in a 2D femur model and a partial bone defect in a 3D femur model. The time-dependent mechanobiology-based scaffolds showed better long-term inward bone-growth results than those based on optimal initial mechanical stimulation alone. Nevertheless, they did not consider the actual cell invasion of pores or the differences in tissue types (e.g., fibrous tissue vs. cartilage). Perier-Metz et al. (2021) also indicated that the initial mechanical conditions optimized for scaffolds could not ensure optimal predictive bone regeneration results, and the mechanical properties calculated immediately after scaffold implantation could not be used as an indicator of successful bone regeneration.

Osteogenic differentiation of MSCs plays a key role in bone regeneration. The differentiation behavior of MSCs is affected by microenvironmental factors such as matrix hardness and local oxygen tension (Burke and Kelly, 2012). Bone-grafting materials serve as an artificial microenvironment to replace ECM in bone defect areas and should provide beneficial mechanical signals and oxygen conditions to stimulate osteogenic differentiation of MSCs. Cells have the ability to sense differences in the mechanical properties of matrix materials and respond by modifying their adhesion shape and differentiating into distinct cell lineages. MSCs tend to differentiate into nerve cells and chondrocytes in soft substrates (0.1–1.0 kPa) and moderately hard substrates (50–100 kPa), respectively, but into osteoblasts in hard substrates (>100 kPa) (Ansari et al., 2022). Thus, some researchers consider that bone-grafting material simulating the hard matrix environment of bone is conducive to bone formation (Ansari et al., 2022).

4.3.3 Vascularization and innervation of bone grafts

An adequate oxygen supply favors osteogenesis. When damage occurs in bone, vascularization plays an important role in supporting the growth and activity of osteogenic cells. Rapid and sufficient vascularization of bone grafts provides high oxygen tension, which is conducive to osteogenic differentiation of stem cells and direct osteogenesis. After implantation, new bone is usually formed first and is most abundant at the periphery of the bone-grafting materials, whereas osteogenesis inside is slower and less abundant. This is related to osteoblast migration and inner vascularization. Therefore, a successful repair of a bone defect cannot be separated from a sufficient blood supply, which explains why angiogenesis has recently attracted increasing attention in relation to bone regeneration (Kanczler and Oreffo, 2008; Kim et al., 2015). Inadequate blood supply restricts the supply of nutrients and oxygen to osteoblasts, which will be irreversibly damaged after about 2 h of ischemia (James and Steijn-Myagkaya, 1986). To access enough nutrients and oxygen, cells need to be within 200 μm of a vessel, which is commonly considered the diffusion limit of nutrients and oxygen within tissue (Rouwkema and Khademhosseini, 2016). Clinically, large-scale bone defects caused by tumors, trauma, or other factors are not uncommon. The size of such defects hinders vascular ingrowth of a non-vascularized bone grafting and leads to poor nutrient/oxygen delivery, cell death, and core necrosis. A lack of blood vessels also affects the transmission pathways of bone precursor cells and signaling molecules involved in bone repair (Rouwkema et al., 2008). A variety of approaches have been examined to promote adequate vascularization, such as combining biomaterials with vasculogenic cytokines (such as vascular growth factors) or cocktails of growth factors (such as platelet-rich plasma), co-culture with vascular cells (such as endothelial cells and blood vessel smooth muscle cells) that can produce vasculogenic growth factors to achieve early vascularization, and designing specific structures conducive to migration of vascular endothelial cells and vascular ingrowth. A large pore size and high porosity allow faster vascularization, which is conducive to osteogenic differentiation of stem cells and leads to direct osteogenesis (Santos and Reis, 2010). To enhance vascularization and bone formation in bone grafts, an aperture of over 300 μm is usually recommended (Hutmacher et al., 2007; Wang et al., 2022), but there seems to be no marked increase in vascularization when the pore size exceeds 400 μm (Bai et al., 2010). Additionally, an excessive increase in pore size leads to a decrease in the cell-to-cell contact ratio, and cells perceive their surfaces as 2D substrates rather than 3D porous structures. The emerging 3D biological printing technology can be used to directly fabricate biological materials mixed with living cells, proteins, or growth factors, which makes vascularized 3D tissue-engineered bone a possible approach for bone defect treatment (Bendtsen et al., 2017; Xu et al., 2018; Yazdanpanah et al., 2022). For instance, Shen et al. (2022) used in situ 3D bio-printing techniques to deposit vascular endothelial cells onto the inner surface of a stent interconnecting channel. They noted a uniform distribution of endothelial cells within the channel, characterized by enhanced proliferative efficiency, which subsequently facilitated the formation of vascular networks via proliferation and migration in in vitro assays. Experiments in animal bone-defect models demonstrated that these vascularized bone grafts showed good performance in promoting new bone formation. Moreover, developing a highly vascularized tissue-engineered bone graft by prevascularization with the insertion of blood vessels into the scaffold has become a useful method for repairing large bone defects. Wang et al. (2010) reported the construction of segmental defects in the femurs of rabbits, which were then implanted with bone grafts (MSCs/β-TCP scaffold) prevascularized with the insertion of femoral vascular bundles into the side groove of the scaffold. They found that the prevascularized bone grafts improved new bone regeneration and vascularization compared to the non-vascularized group, and this effect was associated with the up-regulated expression of endogenous vascular endothelial growth factor (VEGF).

Several previous studies in tissue engineering have shown that, in addition to vascularization, innervation plays a crucial role in promoting tissue-engineered bone formation (Fan et al., 2014; Wu et al., 2015). The nervous system is a significant supporting tissue for the normal physiological activities of bone tissue (Wang et al., 2020; Sun et al., 2023). The peripheral nervous system in bone not only transmits signals generated from internal/external stimuli to the central nervous system, but also secretes neuropeptides that stimulate the neuropeptide receptors of osteocytes to regulate osteogenic differentiation (Marrella et al., 2018). The literature suggests a strong relationship between bone remodeling and innervation during fracture healing (Wang and Su, 2021). The insufficiency or total lack of peripheral innervation may lead to failed fracture healing, manifesting as delayed healing or nonunion (Marrella et al., 2018). Therefore, the development of novel innervated biomaterials promoting more efficient and effective bone regeneration and osteointegration is urgently needed. Recently, effort has been put into developing new bone-grafting materials with appropriate properties that provide a favorable microenvironment to induce neuronal differentiation and proliferation of stem cells. However, compared with osteogenic cells, the differentiation and growth of neurogenic cells require distinct microenvironments. A soft substrate (0.1–1.0 kPa) is more conducive to neuronal differentiation of stem cells, and a biomaterial surface with a lower level of roughness and a small pore size promotes the growth of neuronal cells. Therefore, identification of the optimal material characteristics is important for facilitating neural tissue formation and supporting bone regeneration. Composite materials designed with different stiffness, roughness, and porosity of materials inside can be considered. Moreover, it may also be feasible to integrate autologous nerve tissue into bone grafts to promote bone formation through innervation. Wu et al. (2015) integrated sensory nerves into β-TCP/cell constructs, which were used to repair critical-size femur defects of rabbits. Rabbits with the integrated constructs produced better bone formation than the implanted group without integration of sensory nerves. They found that the pre-implanted sensory nerve could grow rapidly into the scaffold and increase the expression of calcitonin gene-related peptide, which is conducive to bone defect healing. Their study suggested that it is feasible to implant sensory nerves to enhance the neurotization of tissue-engineered bone, resulting in better osteogenesis.

4.3.4 Approaches to simulation of endochondral ossification

In recent years, strategies that use the developmental process of endochondral ossification (ECO) to improve bone regeneration have received increasing attention in the field of bone tissue engineering (Fu et al., 2021). There are two main types of osteogenic mechanisms: intramembranous ossification (IMO) and ECO. It is well established that ECO is a critical process in the development and healing of long bones, which relies on the establishment of a cartilage template (Thompson et al., 2015). In the ECO process, MSCs differentiate into chondrocytes thereby forming cartilage templates. This is followed by hypertrophic differentiation and secretion of angiogenic and osteogenic factors in the chondrocytes, leading to cartilage calcification, penetration of blood vessels, and new bone formation (Knuth et al., 2019). However, traditional tissue-engineered bone grafting has typically focused on directly promoting MSCs to differentiate into osteoblasts, thereby accelerating bone formation through the IMO pathway, which is unfavorable for graft vascularization and might lead to core necrosis. Nowadays, it is recognized that the new regenerative strategies mimicking the natural bone development process are beneficial to natural induction of vascularization at the graft site. Bone grafts based on the ECO pathway induce cell migration and vascularization by angiogenic and osteogenic factors secreted from hypertrophic chondrocytes. Meanwhile, initial hypoxic conditions caused by the destruction of blood vessels in the bone loss area favor the formation of a cartilaginous template instead of direct IMO (Dennis et al., 2015). The subsequent stages involve cartilage hypertrophy, vascular ingrowth, matrix calcification, and other sequential processes that create a conducive environment for final bone formation. The feasibility of this biological strategy has been demonstrated in several studies through the implantation of engineered cartilage templates into large bone defects. The strategy based on developmental engineering for recapitulating ECO typically involves two procedures: engineering a cartilaginous intermediate in vitro and then implanting the cartilage template into the bone defect to induce bone regeneration (Dennis et al., 2015). Bernhard et al. (2017) developed tissue-engineered hypertrophic chondrocyte constructs by seeding adipose-derived stem cells into decellularized trabecular bone scaffolds. The constructs were cultured for five weeks, including differentiation in chondrogenic medium (two weeks) and maturation in hypertrophic medium (three weeks). Then, they implanted the grafts into critical-size femoral defects in rats, and found that hypertrophic chondrocyte grafts bridged 7/8 of bone defects after 12 weeks, compared to only 1/8 for osteoblast grafts and 3/8 for acellular scaffolds. The chondrocyte grafts were associated with extensive bone remodeling, bone marrow formation, and the presence of pro-regenerative M2 macrophages. Pitacco et al. (2023) used 3D bioprinting to design hypertrophic cartilaginous templates capable of directing endochondral bone formation in critical-size rat bone defects. This process involves the incorporation of human MSCs into PCL/fibrin-based bioink constructs and the use of in vitro culture regimens to produce engineered cartilage grafts. The implanted early hypertrophic constructs supported higher levels of vascularization and bone formation and lower levels of heterotopic bone formation than the positive controls (BMP-2-loaded collagen-nanohydroxyapatite (nHA) scaffolds). These studies have shown tremendously promising results in critical-sized long bone defect reconstruction in animal models, and suggest the potential value of ECO-based bone grafts in critical-sized bone defect reconstruction.

5 Conclusions

Large bone defect repair in load-bearing bone is a major challenge in clinical settings. Transplantation of natural bone tissue, especially autologous bone transplantation, is the traditional and most widely used treatment method, and its superior clinical effect has been proven over many years of application and follow-up. However, the clinical application of natural bone tissues is constrained by their inherent limitations. In recent years, the intense demand for excellent bone substitutes has driven the development of novel bone graft research. The various novel bone-grafting materials that have so far been developed still have certain deficiencies compared with autografts. However, they do offer advantages that natural bone grafts lack and have promising development prospects. This review summarizes the development strategies for new bone-grafting materials from three angles: substance bionics, structural bionics, and functional bionics (Fig. 3). Achieving “perfect” weight-bearing bone defect repair may not be possible with a single class of biomaterials, and a combination of two or more biomaterials may be a more sensible choice. Bioceramic materials, which exhibit commendable bioactivity and osteoinductivity, hold particular promise. Nonetheless, their suboptimal mechanical properties necessitate combination with other biomaterials that have superior mechanical strength. Metals and selected polymer materials, characterized by their high mechanical strength, exhibit substantial applicative value for the remediation of critical size defects in load-bearing regions. Improving their bioactivity and avoiding stress shielding are key to ensuring their long-term stable application in vivo. While this review provides a comprehensive overview of various novel bone-grafting materials, it lacks detailed comparisons of their clinical performance because most are still at the laboratory research stage. In the future, the development of materials and techniques will open up broader prospects for the repair and reconstruction of load-bearing bone defects. More clinical research is needed to investigate the success rates, patient outcomes, and complications of novel bone-grafting materials.

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Author contributions

Dan YU performed the conceptualization, project administration, and writing the manuscript. Wenyi SHEN contributed to the project administration and writing the manuscript. Jiahui DAI helped in the editing of the manuscript. Huiyong ZHU contributed to the editing of the manuscript and supervision. All authors have read and approved the final manuscript.

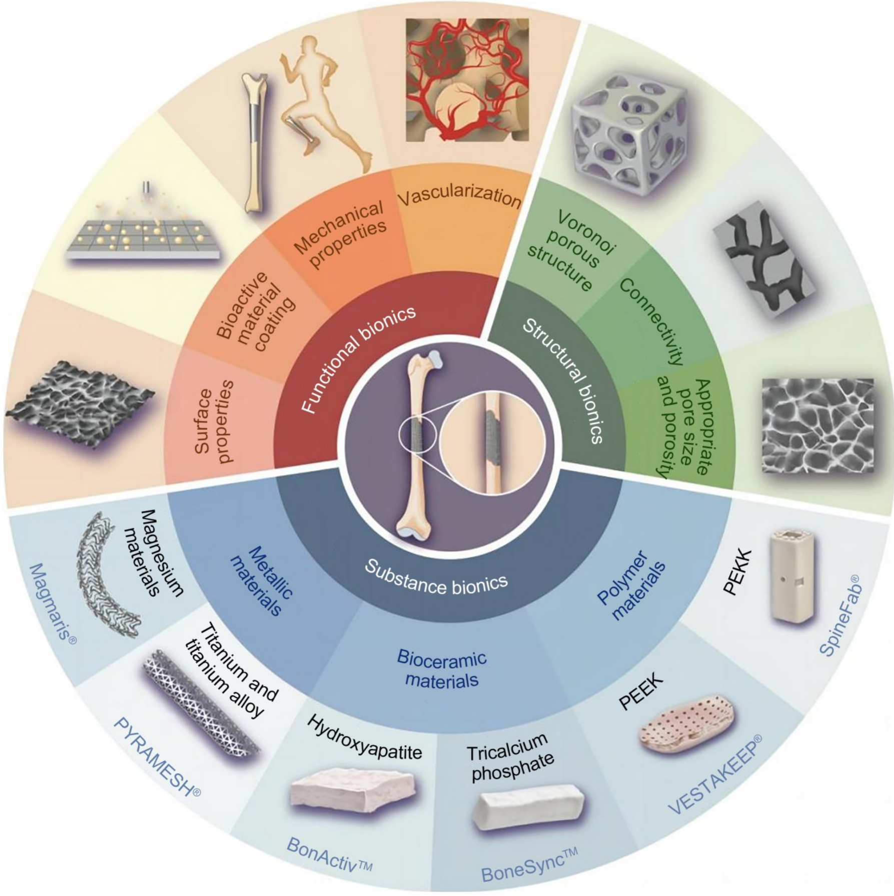

Fig. 3 Development strategies for new bone-grafting materials from three angles: substance, structural, and functional bionics. PEEK: polyetheretherketone; PEKK: polyetherketoneketone.

Compliance with ethics guidelines

Dan YU, Wenyi SHEN, Jiahui DAI, and Huiyong ZHU declare that they have no conflicts of interest.

This review does not contain any studies with human or animal subjects performed by any of the authors.

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